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cultures. The abundance of GFP transgene expressing cells was quantitated by indirect fluorescent microscopy at 4, 8, 22, 30, and 40 days. The results represent the mean (+/-SEM) of 4 independent experiments for each condition.

Please replace the paragraph beginning at page 12, line 13 with the following paragraph:

Figures 7A and 7B. [Figure 7.] Proteasome inhibitors differentially augment rAAV transduction from the apical or basolateral surfaces of airway epithelia. The efficiency and time course of rAAV transduction were evaluated in polarized airway epithelial cultures following infection with rAV.GFPori3 (MOI=10,000 particles/cell) in the presence or absence of 40 μM LLnL. Transgene expression was monitored by indirect fluorescence microscopy at the indicted time points by quantifying the mean number of GFP positive cells per 10 x field (mean +/- SEM of 3 independent samples for each time point). The effect of LLnL treatment was compared between matched sets of tissue samples at each time point following infection from the apical (Figure 7A [Panel A]) or basolateral (Figure 7B [Panel B]) surfaces. The photomicrographs on the right side of each figure [panel] illustrate representative 20 x fields for the 3 and 22 day post-infection time points.

Please replace the paragraph beginning at page 13, line 32 with the following paragraph:

Figures 11A and 11B. [Figure 11.] Optimization of LLnL-enhanced transduction in polarized bronchial epithelia. Differentiated transwell cultures were infected with AV.GFP3ori (10,000 particles/cell) from either the basolateral (Figure 11A [Panel A]) or the apical (Figure 11B [Panel B]) surface with the indicated treatments involving LLnL and/or EGTA. All infections were carried out for 24 hours and GFP transgene expression was monitored by indirect fluorescent microscopy at the indicated times. Data represent the mean (+/- SEM, N=6) for each experimental condition. Experiments were performed in triplicate on transwells derived from samples obtained from two different patients. The following conditions were evaluated for basolateral infection in Figure 11A [Panel

A]: 1) single infection with AV.GFP3ori alone (black line), 2) single infection with AV.GFP3 ori in the presence of 40  $\mu$ M LLnL (solid purple line), 3) single infection with AV. GFP3 ori in the presence of 40  $\mu$ M LLnL followed by repeated 5 hour exposure to 40  $\mu$ M LLnL in the basal compartment culture medium every 3rd day thereafter (solid red line), 4) single infection with AV.GFP3ori in the presence of 40  $\mu$ M LLnL followed by the continued exposure to 40  $\mu$ M LLnL in the basal medium after rAAV was removed (solid green line), and 5) repeated infection with AV.GFP3 ori on day 1 and 15 in the presence of 40  $\mu$ M LLnL for 24 hours at the time of infection (dashed blue line). The following conditions were evaluated for apical infection in Figure 11B [Panel B]: 1) single infection with AV.GFP3ori alone (solid black line); 2) single infection with AV.GFP3ori following pretreatment with 3 mM hypotonic EGTA prior to the viral infection (solid purple line); 3) single infection with AV.GFP3ori in the presence of 40 μM LLnL (solid green line); and 4) single infection with AV.GFP3ori in the presence of 40 uM LLnL following pretreatment with 3 mM hypotonic EGTA prior to the viral infection (solid red line).

Please replace the paragraph beginning at page 14, line 25 with the following paragraph:

Figures 12A and 12B. [Figure 12.] Binding and uptake of S<sup>35</sup>-labeled AV.GFP3ori in fully differentiated human bronchial epithelia. The ability of polarized bronchial epithelia to bind and internalize virus from the apical or the basolateral surfaces was quantified using S<sup>35</sup>-labeled rAAV. The binding assay was performed after incubation with virus at 4°C for 1 hour, followed by repeated washing in PBS. The combined bound and internalized virus was quantified following incubation with virus at 4°C for 1 hour, and subsequent incubation at 37°C for 2 hours and 24 hours. Nonspecific background binding of radiolabeled virus was determined in parallel studies on collagen coated empty chambers not seeded with bronchial cells. Background counts (averaging 15.67 +/- 5.17 cpm/well) were subtracted from experimental sample counts prior to analysis. Data in the right side of each figure [panel] is presented as the net cpms of bound/internalized virus (raw counts minus background counts of empty transwells).

The results represent the mean (+/- SEM) of 6 independent transwells for each condition. Experiments were performed in triplicate from two independent tissue samples. The significance of the differences between each pair of samples (with or without LLnL) was evaluated using the Student's t-test and p-values are provided in brackets above the data for each condition. To correlate uptake of radioactive virus with the functional expression the rAAV encoded transgene, GFP expression from the same set of samples was quantified at 24 hour post-infection by indirect fluorescent microscopy. The results (Mean +/- SEM, N=6) are presented as a bar graph on the right side [of each panel].

Please replace the paragraph beginning at page 16, line 14 with the following paragraph:

Figures 15A-C. [Figure 15.] Examination of rAAV endocytosis by Southern blot analysis of viral DNA. Hirt DNA from AV.GFP3ori infected or mock infected (Lanes 1 and 7 in both Figures 15A and 15B [Panels A and B]) human bronchial epithelia were extracted for a direct examination of viral genomes by Southern blotting against a P32labeled EGFP probe. Figure 15A [Panel A] depicts viral binding studies in the presence and absence of LLnL with or without EGTA treatment prior to apical or basolateral infection for 1 hour at 4°C. Cell surface-bound virus was completely removed by trypsin (Figure 15A [Panel A], lanes 2 through 6). To determine the amount of the surfacebound rAVV, cells were infected with AV.GFP3ori for 1 hour at 4°C and were not treated with trypsin prior to Hirt DNA extraction. Panel A: lane 8: apical AAV infection; lane 9: apical AAV infection in the presence of LLnL; lane 10: cells were pre-treated with hypotonic EGTA prior to apical infection in the presence of LLnL; lane 11: basolateral infection; lane 12: basolateral infection in the presence of LLnL. Figure 15B [Panel B] depicts the results of studies evaluating rAAV internalization from either the apical or the basolateral surface in the presence or absence of LLnL, and the internalization from the apical surface after combined treatment with hypotonic EGTA and LLnL. To detect the net amount of the internalized viral genome, all samples in Figure 15B [Panel B] were treated with trypsin just before Hirt DNA was harvested. The extent of the internalized virus at 4 hours (Figure 15B [Panel B], lanes 2 through 6) and

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24 hours (Figure 15B [Panel B], lanes 8 through 12) incubation at 37°C after infection is represented by the intensity of the 1.6 kb single stranded viral genome band. Figure 15B [Panel B]; lane 2; apical AAV infection for 4 hours; lane 3: apical AAV infection in the presence of LLnL for 4 hours; lane 4: cells were pre-treated with hypotonic EGTA prior to apical infection in the presence of LLnL for 4 hours; lane 5: basal infection for 4 hours; lane 6: basolateral infection in the presence of LLnL for 4 hours; lane 8: apical infection for 24 hours; lane 9: apical infection in the presence of LLnL for 24 hours; lane 10: cells were pre-treated with hypotonic EGTA prior to apical infection in the presence of LLnL for 24 hours; lane 11: basolateral infection for 24 hours; lane 12: basolateral infection in the presence of LLnL for 24 hours. Figure 15C [Panel C] compares the effect of LLnL/EGTA on rAAV genomes at 2, 10, 30 days following a 24 hour infection from the apical (lanes 1, 2, 5, 6, 10, 11 and 12) and basolateral (lanes 3, 4, 7, 8, 9, 13 and 14) membranes. Treatment conditions are noted above each lane; transwells were not treated with trypsin prior to harvesting Hirt DNA. An additional control included coinfection with Ad.d1802 (MOI=500 part/cell) to demonstrate replication form monomers (lane 9, 4.7 kb). It should be noted that different exposure times were used for the three different panels in Figure 15C [C] (lanes 1-4, 3 hours; lanes 5-8, 15 hours; lanes 10-14; 12 hours). Matched DNA samples from uninfected cultures did not demonstrate detectable hybridization (data not shown).

Please replace the paragraph beginning at page 17, line 21 with the following paragraph:

Figures 16A-D. [Figure 16.] Modification of the viral ubiquitination state facilitates rAAV transduction. Similar to the polarized human airway cells, rAAV transduction in human primary confluent fibroblasts was also augmented by tripeptide proteasome inhibitors. 80% confluent human primary fibroblasts were infected with AV.GFP3ori at an moi of 1000 DNA particles/cell. Figure 16A [Panel A] depicts GFP transgene expression in the absence (left photographs) and presence of 40  $\mu$ M LLnL (right photographs) at 96 hours post-infection. Similar effects were achieved with 4  $\mu$ M Z-LLL (data not shown). Top and bottom panels represent bright field and FITC-channel

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fluorescent photomicrographs, respectively. The mean (+/-SEM, N=3) percentage of cells transduced with rAAV, as measured by FACS sorting of GFP expressing cells, is presented in the bar graph of Figure 16B [Panel B]. Figure 16C [Panel C] demonstrates the identification of the ubiquitinated AAV capsid proteins (marked by arrowhead) 6 hours after infection of primary confluent fibroblasts. In this study, rAAV from infected cells was first immunoprecipitated with anti-VP1,2,3 (AAV-2 capsid) monoclonal antibody followed by Western blot detection of ubiquitin side chains using an antiubiquitin monoclonal antibody. The two major background bands migrating at approximately 65 and 25 kd represent heavy and light chain antibody subunits which cross-react with secondary antibodies. Additionally, the equal intensity of lower molecular weight cross-reactive bands (30-40 kd) serve as internal controls for equal loading of protein. Figure 16D [Panel D] demonstrates augmentation of rAAV transduction in polarized airway epithelia by inhibitors of ubiquitin E3 ligase. Epithelia were infected with AV.GFP3ori (10,000 particles/cell) from the basolateral surface following treatment with ubiquitin ligase inhibitor dipeptides (0.2 mM H-Leu-Ala-OH and 0.2 mM H-His-Ala-OH). Results demonstrate the mean (+/- SEM, N=3) number of GFP expressing cells per 10x field at 1 and 15 days post-infection.

Please replace the paragraph beginning at page 18, line 15 with the following paragraph:

Figures 17A-F. [Figure 17.] Persistent induction of rAAV mediated gene transfer in mouse conducting airways by proteasome inhibitors. Figures 17A-C [Panels A-C]) Recombinant AV. Alkphos ( $5 \times 10^{10}$  particles) was administered to mouse lung either as virus alone in PBS or virus in combination with 40 μM LLnL in PBS. Virus was directly instilled into C57Balb/c mice trachea with a 30 G needle in a total volume of 30  $\mu$ l. To insure the spread of the virus in mouse lung, 50  $\mu$ l air was pumped into lung through the same syringe immediately after virus was administered. Ninety days after infection, lungs were harvested intact and fixed in 4% paraformaldehyde followed by cryosection. AAV-mediated transgene expression was evaluated by 10  $\mu$ m tissue sections staining for heat-resistant alkaline phosphatase. Figure 17A [Panel A]: infection with AAV alone;

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Figures 17B and 17C [Panels B and C]: infection with AAV supplemented with 40 M of LLnL. Figures 17D-F [Panels D-F]) 6 week old BALB/c mouse (N=3 animals in each group) were infected with 5 x  $10^{10}$  DNA particles of AV.LacZ in the absence or presence of 400  $\mu$ M Z-LLL by nasal instillation. Representative examples of histochemical staining for LacZ expression in large bronchioles 150 days post-infection are shown in Figures 17D and 17E [Panel D and E]. The right and left sides of each panel represent Nomarski and bright field photomicrographs, respectively. The 100  $\mu$ m scale bar applies to all photomicrographs. The mean (+/-SEM) percentage of LacZ expressing epithelial cells at various levels of the airway was quantitated using the morphometric procedures outlined in the methods, and the analysis represents results from three independent animals for each group (Figure 17F [Panel C]).

Please replace the paragraph beginning at page 19, line 4 with the following paragraph:

Figures 18A-D. [Figure 18.] Cy3-labeled rAAV infection in Hela cells. Hela cells were infected with Cy3-labeled AV.GFP3ori at an MOI of 500 particles/cells for 90 minutes at 4°C in the absence of serum. Cells were then washed and either directly fixed in 2% paraformaldehyde for 10 minutes or incubated at 37°C for an additional 60 or 120 minutes prior to fixation. Prior to viral infections, cells were incubated for 30 minutes in 1 μM 5-chloromethylfluorescein diacetate (Cell Tracker<sup>TM</sup> Green CMFDA, Molecular Probe) to allow for visualization of cells and labeled virus in dual fluorescent channel images. Representative confocal image of cells infected for 90 minutes at 4°C followed by a 60 and 120 minute incubation at 37°C are shown for both Cy3 and dual Cy3/FITC channels (Figure 18A [Panel A]). The nuclei in dual channel images are marked by Nu. The confocal images shown were merged from three 0.5  $\mu$ m layers taken within the central region of the cell. Figure 18B [Panel B] depicts non-confocal images with Nomarski and Cy3 channels for cells infected for 90 minutes at 4°C (left side of Figure 18B [Panel B]) followed by a 120 minute incubation at 37°C (right side of Figure 18B [Panel B]). Virus binding at 4°C localizes to the surface membrane of the cell. With increased incubation time at 37°C, virus was translocated to the nuclear membrane.